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Effekten av tillsatt nitrat och sulfat i dieten på metanproduktion hos nötkreatur

Karolin Bäckman

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Abstract

A growing population together with a change in food habits to a more diverse diet containing more animal products such as meat and milk makes the possibility to produce and sell products of cattle a growing market. And at the same time there is an ongoing debate about global warming, where raising cattle is questioned due to their high production of enteric methane. To be able to raise animals in a more environmental friendly way and at the same time improve productivity in a developing country such as Vietnam would be favorable. The use of feed additives with high electron affinity could decrease the methane production and at the same time improve the gross energy digested. Nitrate and Sulfur are both good electron acceptors, but due to its toxic effects the use of nitrate in feed has been neglected. Recently scientists have recorded that animals adapted to high amounts of nitrate can overcome these toxic effects. This present study used 4 male Yellow cattle which were fed a basal diet added with either of Urea, Urea + Sulfur, Nitrate or Nitrate + Sulfur in a Latin square design. There was a significant lower ratio of CH_4/CO_2 with the treatment of Nitrate + Sulfur compared with the treatment of Urea which indicate a lower eneric methane production. The study also showed a significantly lower feed intake of the treatments Nitrate, Urea + Sulfur and Nitrate + Sulfur compared with the Urea treatment. Due to a low animal number the study can only be seen as a pilot study. Furthermore, it could also be discussed if the use of nitrate is likely to be used of small-scale farmers due to the high risk of poisoning if used inaccurate.

Sammanfattning

Med en växande befolkning samtidigt som förändringar av matvanor sker till en mer varierad kost, bestående av mer animaliska produkter såsom kött och mjölk, bidrar det till en ökande marknad för animaliska produkter. Samtidigt pågår diskussionen om den globala uppvärmningen, där idisslarna ifrågasätts på grund av dess höga produktion av enterisk metan. Att kunna föda upp djur på ett mer miljövänligt sätt och på samma gång förbättra produktiviteten i ett utvecklingsland som Vietnam skulle vara gynnsamt för alla parter. Användning av fodertillsatser med hög elektronaffinitet kan minska metanproduktionen och samtidigt öka energiintaget. Nitrat och sulfat är båda bra elektronacceptorer, men på grund av nitrats toxiska effekter har användningen av nitrat i foder förbisetts. Men under de senare åren har forskarna visat att djur som är tillvanda till stora mängder nitrat kan övervinna dess toxiska effekter. I den här studien användes 4 handjur av rasen Yellow cattle som utfodrades med en basdiet kompletterad med något av ; urea, urea + svavel, nitrat och nitrat + svavel i en latin square design. Studien visade en signifikant lägre kvot av CH_4/CO_2 vid utfodring av Nitrat + Sulfat jämfört med Urea + Sulfat vilket indikerar lägre enterisk metanproduktion. Studien visade även ett signifikant lägre foderintag för behandlingarna med nitrat, urea + svavel och nitrat + svavel jämfört med urea behandlingen. På grund av ett lågt djurantal så kan studien endast ses som en pilotstudie. Det kan också diskuteras om det är rimligt att nitrat ska användas av småskaliga bönder, då risken för nitritförgiftning av djuren är överhängande vid felaktigt användande av nitrat.

Contents

Introduction

With a growing population and economy in developing countries the demands for both dairy products and meat have grown substantially. Due to increased urbanization and income levels, the diet have changed to a more protein rich and varied diet (Steinfeld *et al.*, 2006). More people is moving to the cities and changing their food habits from local options often rice, locally produced vegetables, fruits and small quantities of animal products to a more divided diet, with a greater portion of animal products, fat and sugar. This makes the livestock production to be one of the most growing sectors within the agricultural sector (FAO, 2012). The increase in consumption of livestock products is mainly seen in China and South East Asia (Steinfeld *et al.*, 2006). In order to meet the growing demand for livestock products, this region will need to increase import and develop their local livestock production and at the same time the discussion about global warming is globally of high priority. The anthropogenic greenhouse gas emissions need to be lowered to not risk a substantial rise of the temperature of the earth (IPCC, 2007).

According to Steinfeld *et al.* (2006) the livestock production is contributing with 18 percent of the total anthropogenic greenhouse gas emissions, where enteric fermentation and manure represent over 80 percent of the total agricultural methane emissions. It is therefore necessary to develop tools to mitigate methane emissions. These tools can be divided into three groups: Improved feeding practices, usage of feed additives or specific agents, management changes and breeding (Smith *et al.*, 2008). Management changes and breeding are long-term practices. Breeding for mitigating greenhouse gas production and improved productivity (Wall *et al.*, 2009) are the long-term choices in breeding. Breeding for mitigating greenhouse gases is today difficult due to the complicatedness of measuring the methane production as a breeding value for each individual. Animals in the developed world produce less methane per produced unit compared to the undeveloped part of the world. This is due to an improved efficiency in feed production, the nutritional value of the feed and also because of good breeding strategies for improved efficiency and health of the animals. The feeding strategies are consisting of a more intense production due to increased concentrate rations (Smith *et al.*, 2008). Feeding more concentrate results in faster growth and the animals can therefore be slaughtered at a younger age. Feed additives and other agents are often used to suppress the methanogenesis. Such compounds can be vaccines against methanogenic bacteria, probiotics such as yeast, but also compounds that are banned in EU such as bST and antibiotics for mitigating methane emissions. Feed additives can also be substrates with a high electron acceptor capability. One substrate is Nitrate, a high electron acceptor reduced to ammonia that can be used as energy source for the animal, and thereby reduce the methane production (Leng & Preston, 2010).

The aim of the study was to investigate methane emission from beef cattle when fed a diet supplemented with nitrate and sulfur. The hypothesis was that nitrate and sulfur supplementation would have a mitigating effect on the methane production. Using both nitrate and sulfur, as feed additives would give an additive effect on the methane mitigation.

Beef production in Vietnam

Livestock such as cattle are important for the livelihood in the rural areas (Zeleke *et al.*, 2006). Cattle does not only contribute with meat and milk products to farmers they also provide farmers with manure to fertilize the crops, draught and also provide a small and important income when selling products on the market. Both the income from the animal and the animal itself can be a major part of the household economy (de Haan *et al.*, 2001).

Because of the high value of the cattle, is it an important tool for the rural people to get out of poverty.

Beef production in Vietnam is mainly found in smallholder farms, often with a mixed production of beef and vegetables (Huyen, 2011). Approximately 10 % of the beef production is raised by commercial undertaking. The most common breeds are the *Bos indicus* type, such as Yellow cattle, Laisind, Draugthmaster and Brahman. Laisind is a cross breed originated from Red Sindhi and Yellow cattle. These breeds of *Bos indicus* are more resistant against parasites (Maryam *et al.*, 2011), heat and drought and can survive on feeds with low nutritional value compared with *Bos taurus* (Berman, 2011).

Beef cattle in Vietnam are generally fed on common grasslands and the feeding strategies are different in the different regions. In low land areas cattle are often grazing along roadsides and field edges, they are also fed crop residues such as rice straw and small amounts of cultivated elephant grass (*Pennisetum purpureum).* In highlands the grazing areas are bigger and they also use forage pasture, therefore they have an absent feed cost. During dry season, during periods with shortage of feed, the animals are left to graze on the harvested fields. Larger farms often feed their cattle with harvested elephant grass and concentrates such as maize meal, Cassava- (*Manihot esculenta)* root meal, leaf meal and foliage. These animals are also often let out on cultivated pasture some hours each day.

Feed production in Vietnam

In tropical areas such as Vietnam Cassava (*Manihot esculenta,* Crantz) is cultivated in the whole country as one of the most important crop. Cassava is a tropical and sub tropical crop that can be used for both feed and food for humans. Cassava is a high reproductive annual crop where both the root and leafs can be used as feed (Wanapat, 2003; Thang *et al.*, 2009). The root contains high levels of starch and is a great source of fermentable energy for the rumen micro-flora whereas leafs contains a high amount of crude protein 20-30%. But cassava does also contain high amounts of Hydrogen Cyanide (HCN), which has an antinutritional effect on the digestibility of the feed but can also cause poisoning in high concentrations (Thang *et al.*, 2010). During sun drying of different parts of cassava the amounts of HCN is reduced with 35-90% (Wanapat, 2003; Thang *et al.*, 2010). Cassava leafs does also contain high levels of tannins, which also have an methane suppressing effect (Tavendale *et al.*, 2005)

Elephant grass (*Pennisetum purpureum)* is cropped and often harvest by the cut-and-carry system in Vietnam. But the grass is rarely sown because of the need to possess land. Elephant grass is a fast growing grass harvested in a maximum interval of 6 weeks and is often fed chopped and fresh to animals (Man and Wiktorsson, 2003).

Rumen fermentation and methane production

During thousands of years, ruminants have evolved a symbiosis with rumen bacteria, to be able to utilize fibrous feedstuff that cannot be digested of monogastric animals. During rumen fermentation of organic matter, volatile fatty acids (VFA), CO_2 and H_2 are produced (Figure 1). These fermenting microbes work symbiotic with the host as metabolic specialists, and their byproducts is used as nutritional compounds, important for the development and health of the host. VFA is formed both by bacteria and Protozoa. Were the protozoa is generally producing acetic and butyric acid while bacteria produces propionic acid (McDonald *et al.*, 2002). Organisms producing Acetic acid are the largest producers of hydrogen (Boadi *et al.*, 2004), and to remain an efficient fermentation and microbial growth in the rumen hydrogen has to be removed (Sjaastad *et al.*, 2003). This is generally done by converting hydrogen and carbon dioxide to water and methane, by methanogenic Archaea. This process allows H_2 producing bacteria to remain their degradation of organic matter due to low H_2 pressure which otherwise would inhibit the degradation (Boadi *et al.*, 2004).

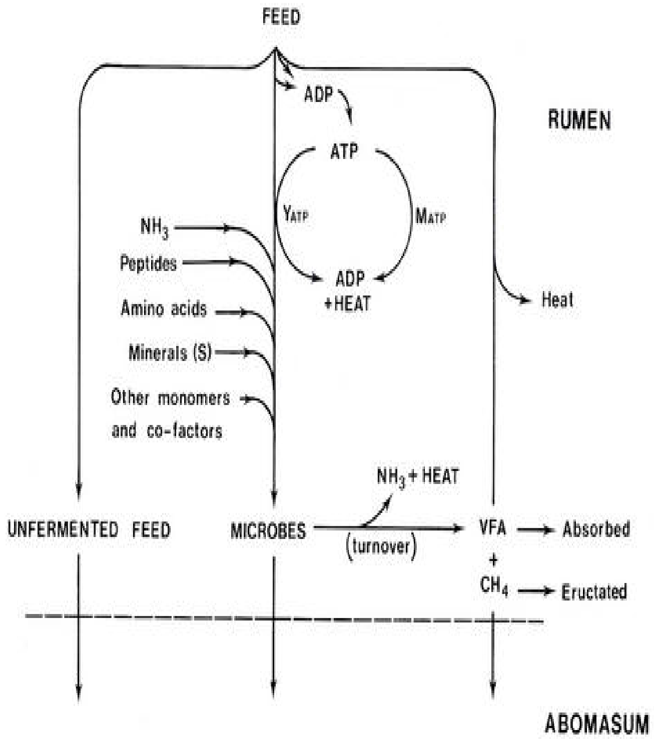

Figure 1. Schematic picture of rumen fermentation (Leng, 1982).

Methane is a colorless, odorless gas and a good potential energy source (Boadi *et al.*, 2004). Approximately 87% of the gas is produced in the rumen and are lost through eructation, which is equal to 2-12% of the gross energy intake (GEI), whereas the other 13% is produced in the large intestine. Reducing the enteric methane and thereby the eructation of methane will mean both lower emissions and a better energy utilization of the feed. Therefore is it important to find a way to reduce the production of H_2 as a method to reduce the methane production (Joblin, 1999) and as the same time increase the gross energy digested (GED). A way to measure the efficiency in the fermentation is to measure the CH_4/CO_2 ratio of the breath (Madsen *et al.*, 2010). The ratio describes the proportion of un-metabolized C in the proportion to the end product CO_2 where the ratio for cattle is 0.08.

Metabolism of nitrate and sulfur

Feeding nitrate to non-adapt animals can lead to accumulation of nitrite in the rumen. Nitrite is an intermediate of the reduction of nitrate to ammonia (Kemp, 1977; Alaboudi & Jones, 1985). Accumulation of nitrite in the rumen leads to absorption of nitrite into blood resulting in oxidation of the ferric iron hemoglobin to methemoglobin (van Zijderveld, 2011). This effect the oxygen transport due to lack of capacity for transporting oxygen, which results in depressed animal performance or during severe cases, death (van Zijderveld, 2010).

The rumen reduction of nitrate to ammonia is more favorable then carbon dioxide reduction (van Zijderveld, 2011). Due to its toxic effects the use of nitrate in feed has been neglected. Alaboudi and Jones (1985) among others found that sheep that were adapted to nitrate got an increased proportion of bacteria capable of reducing nitrite and possibly by using hydrogen from the meathanogenesis. Nitrate as a strong reductant serves as an electron sink and are thereby benefiting the production of acetate and therefore reducing the production of n-butyrate and methane. The reduction from nitrate to ammonia is more energy efficient, then the reduction from carbon dioxide to methane. By using nitrate as an electron sink up to 1 mol of methane can be mitigated from the reduction of nitrate to ammonia (van Zijderveld, 2010). This energy can instead be used as an energy source of the animal instead of lost as a greenhouse gas.

One other alternative electron sink to nitrate could be sulfate (Mathison *et al.*, 1998). It has been reported that also sulphide can act as an electron donor during the nitrite ammonification (Leng & Preston, 2010). Symptoms of toxicities from sulfur are reduced rumen motility, nervous and respiratory distress. These symptoms are caused by hydrogen sulphide converted by the rumen flora (McDonald *et al.*, 2002).

Materials and Methods

The experiment was implemented at the Livestock Feed Research and Trial Station (LFRTS) associated to NIAS, outside Hanoi, between April and August 2011. The LFRTS is located in the Red River Delta at 105°47'E longitude and 21°05'N latitude about 2 m above sea level. The climate is tropical monsoon with a wet season between April and November.

Animals and housing

Four local male Yellow cattle (*Bos indicus*) about 8 months old with an initial body weight of 100 kg ±14 kg was used in the experiment. Before the experiment started the animals were marked with ear tags and treated against parasites with Hanmectin 50 (1 ml/25 kg BW). The animals were held in metabolic cages during the whole experiment and were weight before the adaptation time and collection time and in the end of the collection time (Figure 2).

Figure 2. Animals held in their metabolic cages.

Feeding and management

The basal feed used during the experiment was 3% NaOH treated rice straw, Elephant grass (*Pennisetum purpureum)*, cassava (*Manihot esculenta)* root meal and sun-dried cassava leave meal, molasses. The animals was fed total mixed ration (TMR) four times daily *ad libitum.* Elephant grass was collected daily and cut in pieces of 2-3 cm.

Four different treatments were allocated to the animals complete randomly by a Latin Square Design (4x4) see Table 1. The treatments were arranged, as a 2 x 2 factorial design where the first factor was addition of Ca-nitrate or Urea and the second factor was supplement with or without Na-sulfur. The animals treated with CaNO3 were given 40 g/kg DM feed, animals treated with urea 10 g/kg DM feed and sulfur was fed 0.8% of DM.

Table 1. Treatments and feed ratios

Treatment	Rice straw (g/kg DM)	Elep. Grass (g/kg DM)	Cassava root meal (g/kg DM)	Cassava leaf meal (g/kg DM)	Molasses (g/kg DM)	Ca-nitrate (g/kg DM)	Urea (g/kg DM)	Sulfur (%/kg DM)
Urea	220	60	280	240	200	-	10	0.8
Urea+ Sulfur	220	60	280	240	200	-	10	
Nitrate	220	60	280	240	200	40	-	0.8
Nitrate+Sulfur	220	60	280	240	200	40	-	

The experiment time for each period was 21 days, 15 days of adaptation and 6 days of data collection. During adaptation period the animals were slowly adapted to the treatments, starting with 10% of the total treatment. Each morning before feeding, feed offered and feed residues was weight.

Samplings

During collection time feces and urine were sampled twice per day. Feces samples were then frozen to -20°C. Urine were mixed with 300-400 ml of 10% sulphuric acid to a pH below 2, to preserve the nitrogen. Urine samples were stored in 4°C before analyzing. Feed consumption was recorded daily and feed residue samples were collected every morning before feeding. Feed residue samples were pooled to one sample in the end of the collection period and analyzed.

The method used for measuring methane production was the CO2 Technique, which is based with CO2 as tracer gas (Storm *et al.*, 2012). Measuring the ratio of CH4/CO2 at regular intervals combined with the measuring of total CO2 produced daily, the amount of produced methane can be calculated. The concentration of CO_2, CH_4 and CO_2/CH_4 ratio was measured every second hour during a 24h period on the 6^{th} day, of every data collection period. This was done with GASMET DX4030 portable equipment based on infrared measurements (Gasmet, 2012). The animals were held in the chamber for 15 minutes, and gases were measured the last 5 minutes; see figure 3.

Figure 3. Gasmet chamber.

Chemical Analysis

The feed was analyzed for DM and CP content, urine for nitrogen content and feces for DM and CP content. The method used for analyzing CP was TCVN 4328-86, equal to AOAC method ID 984.13.

Statistical Analysis

Data of CH_4/CO_2 ratio from the collection time was analyzed with GLM procedure in Minitab Software Version 16. The results were compared using the Bonferroni method with a 95% confidence interval.

Results

The ratio of CH_4/CO_2 was significantly higher for the treatment with Urea + Sulfur compared with the treatment of Nitrate + Sulfur ($P<0.05$). The mean ratio of treatment with Urea + Sulfur was 0.054 whereas it is 0.043 with the Nitrate + Sulfur treatment (Figure 4).

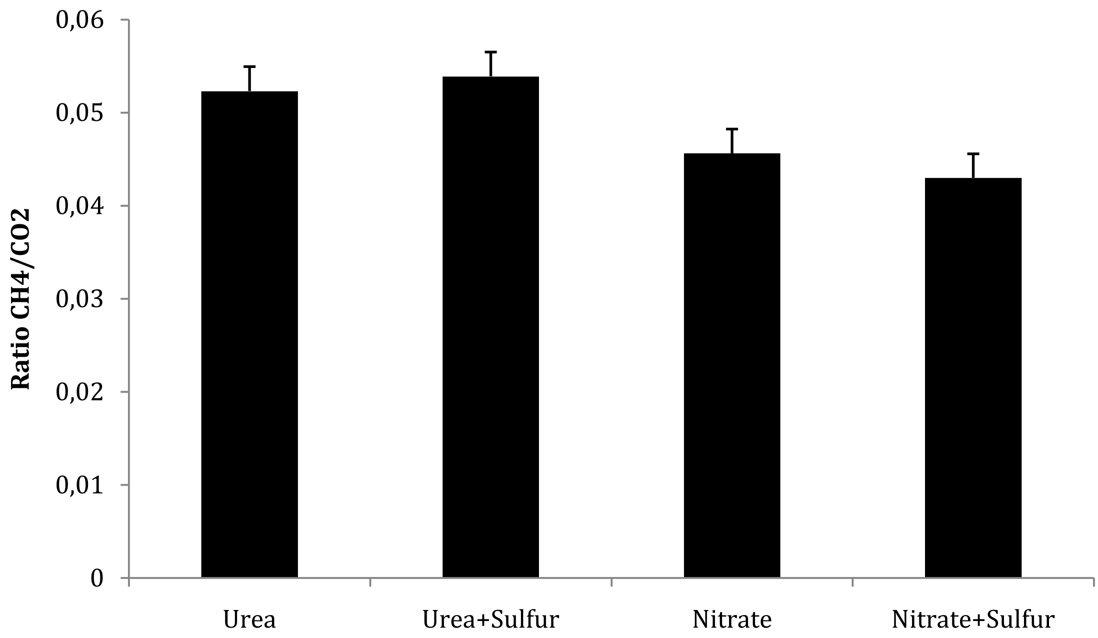

Figure 4. CH4/CO2-ratio for the different treatments, average over periods

Figure 5 shows the treatments with Nitrate and Urea in the diet with or without Sulphate. There is only significant differences between the treatments of Nitrate + Sulfur and Urea + Sulfur. However, figure 5 indicate a difference between the treatment of urea and nitrate.

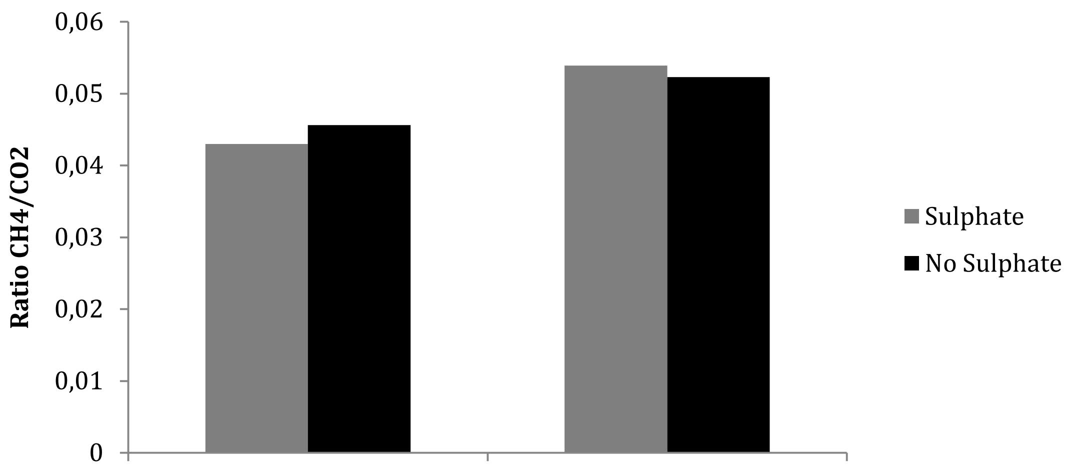

Figure 5. The effect of adding sulfur in diets with Nitrate or Urea.

There was a significant difference on feed intake between treatment with Urea and Nitrate compared to the treatments with additional sulfur (P<0.01). Treatment with urea gave the highest feed intake; thereafter the Nitrate treatment and the treatments containing sulfur gave lower feed intake, (Figure 6). There was a significant difference between all the treatments except from the sulfur treatments.

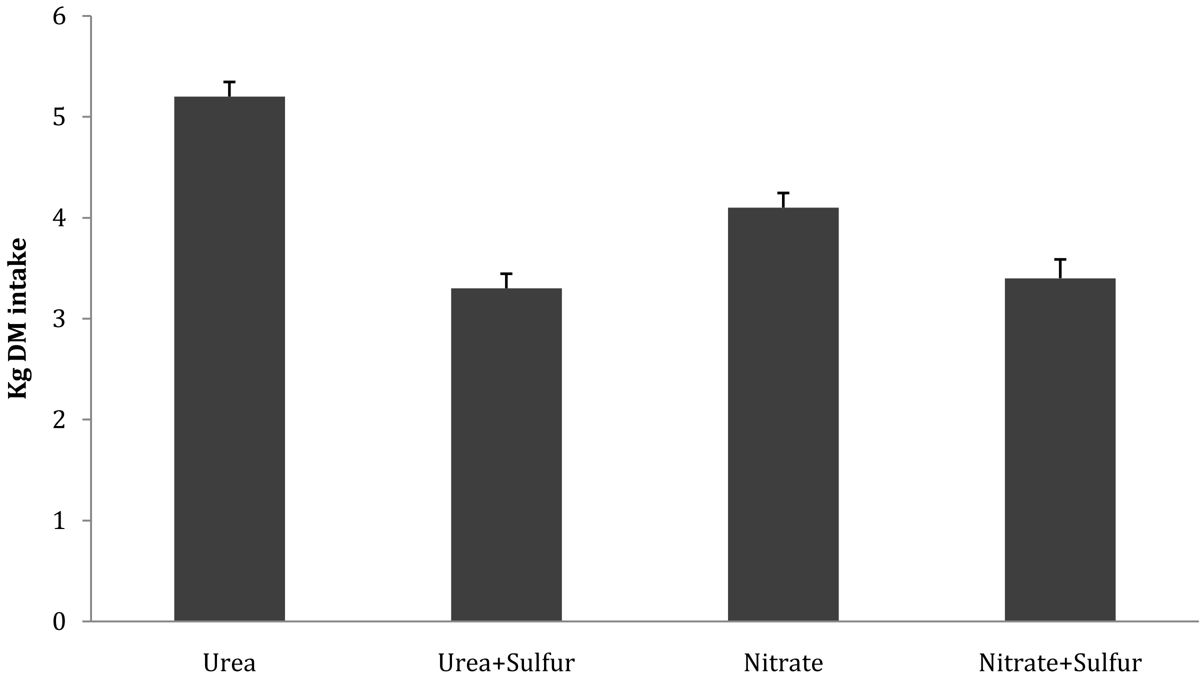

Figure 6. Feed intake for the treatments, average over periods

Discussion

The results showed a significant difference in CH_4/CO_2-ratio between the treatments of Urea + Sulfate and the treatment of Nitrate + Sulfate. The hypothesis was that a use of nitrate as well as sulfur in the diet would have a mitigating effect on the methane production, and the use of both would have an additive effect. This was not the case in this study, adding sulfate to the diet gave the highest ratio. In a study by Van Ziderveld *et al.* (2010) sheep were treated with; urea, nitrate, sulfur and nitrate + sulfur where both the sulfur, nitrate and nitrate + sulfur supplementation had a mitigating effect on the methane production. In the study, 20 sheep were used, which is a much higher number of animals than in the present study. However, Phuong *et al.* (2011) detected in an *in vitro* study an increased methane production when sulfate was used together with urea. The study by Phuong gave as well the highest reduction of methane used with nitrate. These results can be compared with the results in the present experiment. The increased production of methane was explained with the enhanced fermentation stimulated with sulfur supplementation. According to the hypothesis and results of earlier studies (Van Zijderveld *et al.,* 2010; Nolan *et al.,* 2010) supplementation of nitrate gave a small mitigating effect but there were no significant difference in this study. The low number of animals could possibly explain the reason for no significant results.

There was also a significant difference between the diets in feed intake, where the treatments containing sulfur had significantly lower feed intake compared with the urea and nitrate treatment. Considering these results it could be speculated if sulfur has lower palatability and possibly any off-flavor. Also the treatment with nitrate gave a significantly lower feed intake compared with the urea treatment. The reduced feed intake in this treatment could be due to the toxic properties of nitrite. This could be a development due to the toxicity of nitrite. The large variations in feed intake probably have a big influence on the CH_4/CO_2 ratio in the different treatments. There could be speculated that a more homogenous feed intake would give a larger variation between the treatments. The CH_4/CO_2 ratio was in all treatments lower than average ratio on cattle measured at 0.8 by Madsen *et al.* (2010). A reason for a lower ratio can be a result of using the *Bos indicus* that have lower efficiency than the *Bos taurus,* used in the study by Madsen *et al.* (2010). T

Even if the use of nitrate and sulfur has a mitigating effect on the methane production in ruminants, there is still research left to do in this field. However, it could be discussed if feeding with nitrate in developing countries is the best way to go in methane mitigation due to the risk of nitrite poisoning. To use supplement such as nitrate demand farmers to have knowledge of the risk of feeding nitrate to animals. It is also important with a quite long adaption time of the feeding of the animal to develop a micro flora that can reduce nitrate to ammonia. Therefore is it important to feed the animals regularly and no have abruptly changes in the ratios. If the farmer would not have money to buy the feed for a few weeks they have to start over with the adaption time again and cannot start feeding as before as soon they can afford to buy new feeds. The positive aspects of using nitrate in feeds are the nitrogen supply. In tropical feeds the utilization is often lower due to less degradability of the feeds, such as rice straw and elephant grass (Thang *et al.,* 2010). Feeding with nitrate would give a higher proportion of nitrogen in the rations. Small scale farmers with beef production often have a pasture based production, therefore it may be difficult for the farmer to be motivated to initiate concentrate supplementation, as it require more labor and is a more expensive alternative to the pasture.

Conclusions

The methane mitigating effect could not be determined statistically significant but a trend lower CH_4/CO_2-ratio could be due to a slightly lowered methane production when nitrate and sulfur were added. Earlier studies have shown that nitrate have a mitigating effect on the methane production, but further studies are required including a larger number of animals for a more reliable result. The use of nitrate as feed supplement for cattle in small-scale farms in Vietnam, can be discussed due to the risk of nitrite poisoning. The study also showed a significantly lower feed intake when feeding with additives such as sulfur and nitrate. This could have a negative effect on the weight gain. Therefore future research should include different levels of supplemental nitrate to be able to find a suitable balance between methane reducing effect and the animals' health and weight gain.

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